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Previews

Profiling Identifies Precursor Suspects: Notch Family Again!

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Newborn neurons in the adult dentate gyrus pass through several distinct precursor and progenitor classes prior to differentiation. In this issue of *Cell Stem Cell*, Lugert et al. (2010) characterized their strikingly different proliferative behaviors after neurogenic stimuli or aging.

Granule cells of the dentate gyrus of the hippocampus are arguably the only known neurons to be continuously produced in most mammalian species including humans (Kriegstein and Alvarez-Buylla, 2009). However, the magnitude of neurogenesis between species as well as during the lifespan of mammals is remarkably varied. The evolutionary purpose and molecular mechanisms underlying this variation are largely unknown. In the past several years, Notch signaling has emerged as a dominant player in the cell fate and maintenance of neural precursors. In this issue of *Cell Stem Cell*, Taylor and colleagues present the latest addition to this growing body of work (Lugert et al., 2010).

In the past decade, precursors and progenitors in the late postnatal and adult granule cell lineage have been defined by genetic promoter studies and antigenic properties (Seri et al., 2001; Kempermann et al., 2004; Kriegstein and Alvarez-Buylla, 2009). Granule cells are thought to derive from glial fibrillary acidic protein (GFAP)-expressing precursors in the subgranular zone of the dentate gyrus (Seri et al., 2001; Garcia et al., 2004). These precursors termed "Type 1" fall into two classes—radial and horizontal. During development, both radial and horizontal precursors originate from radial glia whose soma translocate into the SGZ, and thus it was assumed that radial glia is the top-level precursors/stem cell population (Seri et al., 2001; Kriegstein and Alvarez-Buylla, 2009). Postnatally, the precise relationship between these two classes has been called into question by elegant lineage tracing experiments (Suh et al., 2007). Nevertheless, a subset, or perhaps all, of these GFAP-expressing precursors have the potential to give rise to more neuronally committed daughter cells (Figure 1) that will continue to proliferate, migrate, and differentiate until finally becoming mature neurons (reviewed in Kempermann et al., 2004).

Taylor and colleagues used genetic models to examine precursor populations in the adult dentate gyrus defined by active Notch signaling. In Hes5::GFP transgenic mice, a reporter for canonical Notch signaling, the authors find that Hes5::GFP labels both radial and horizontal SGZ astroglia much like Notch1 antibodies. FACS sorting demonstrated that this GFP^+ population is a colony forming, self-renewing, bipotent neural precursor population. The authors next inducibly ablated RBP-J in glial cells using GlastCreERT2 knockin mice. RBP-J is the DNA-binding element of the Notch-MAML-RBP-J ternary complex, which leads to transcription of Notch target genes after Notch receptor cleavage. Using this approach, the authors found that ablation of RBP-J led to an almost complete loss of precursors and proliferation in the region. The authors conclude from these experiments that the top-level precursors are regulated by canonical Notch signaling mediated by RBP-J.

Lugert et al. provide a wealth of "apples to apples" comparisons of stimuli and phenomena that alter reactive neurogenesis. Running was found to stimulate proliferation of radial precursors while not altering the ratio of radial to horizontal precursors or the total neural stem cell (NSC) population. In contrast, seizures, induced by intraperitoneal kainic acid injections, activated both populations and expanded the horizontal population. However, as kainic acid is known to cause profound neuronal loss and reactive gliosis in many cases, it might be difficult to distinguish an expansion of neurogenic precursors from astrogliosis related to pathology—especially since horizontal astrocytes would be morphologically similar to reactive astrocytes. Interestingly, kainic acid induced seizures activated both precursor types in aged animals but didn't provoke an expansion of the horizontal population—perhaps indicating a degree of senescence.

An intriguing finding of this study is that Hes5::GFP expression in radial and horizontal glia is essentially unchanged with aging, suggesting that Notch signaling is not sufficient for proliferation and neurogenesis in aged animals. Because neurogenesis is known to decline with age, alternate signaling, or combinations of signaling pathways, such as Wnt, Shh, and FGF, EGF could potentially be more important in regulating the NSC niche in aged animals. Previous work has demonstrated that Notch, Sox2, and Shh appear to preferentially regulate the behavior of the GFAP-expressing precursors (Breunig et al., 2007; Suh et al., 2007; Favaro et al., 2009). Sox2 regulates Shh secretion, which appears to stimulate intracellular Shh signaling through primary cilia in an autocrine fashion (Breunig et al., 2008; Favaro et al., 2009). Conversely, Wnt seems to regulate differentiation of neurons from precursors by inducing expression of later neural differentiation genes such as NeuroD1 through the release of Sox2-mediated repression (Kuwabara et al., 2009).

Future work will be needed to further define the relationship between radial

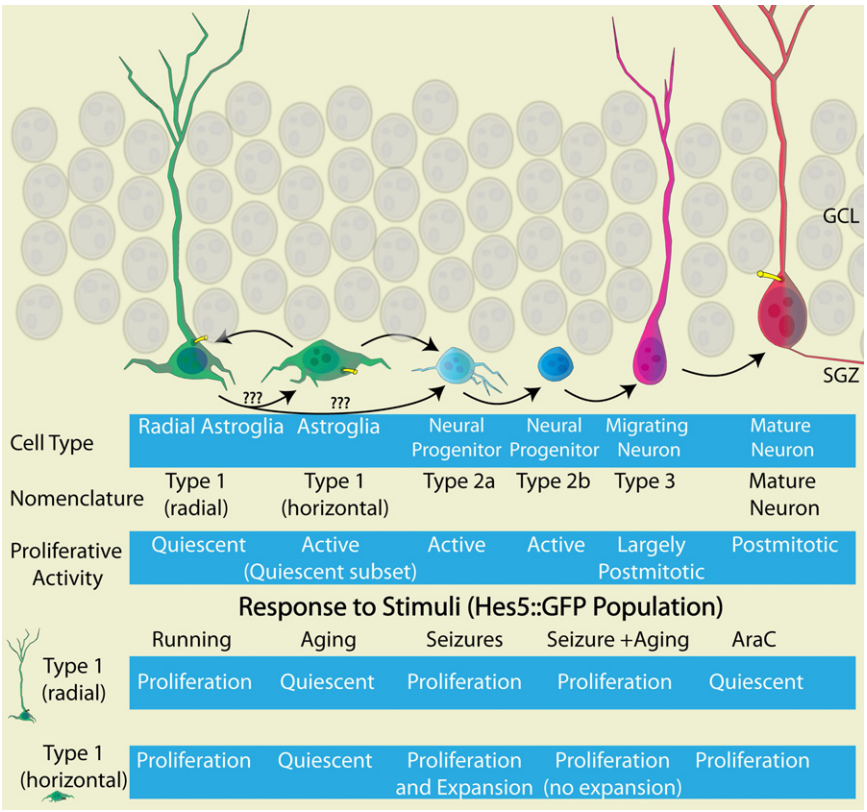

Figure 1. Current View of the Sequence of Neurogenesis from Precursor/Progenitor Cells in the Adult Dentate Gyrus

GFAP$^+$ Type 1 neural stem/precursor cells are believed to divide asymmetrically to give rise to more committed daughter cell types. However, some doubt exists as to the precise lineage relationships between radial and horizontal Type 1 cells. Type 2a cells express Sox2 and Ascl1 but not GFAP$^+$. Type 2b cells begin to express mature neuronal markers such as Dcx. These cell types are believed to undergo symmetric neurogenic divisions. Type 3 cells are migrating neurons that will integrate into the granule cell layer over the course of several weeks in the rodent. Lugert et al. use a surrogate marker of Notch signaling (Hes5::GFP) to label Type 1 cells and show that running, aging, and seizure activity have varied effects on proliferation and expansion of radial and horizontal progenitor populations.

and horizontal glia in the SGZ. Despite their antigenically similar characteristics, it seems safe to assume that the two populations are differentially regulated; however, the findings reported here suggests that Notch signaling is not the key to this heterogeneity. Notably, the findings by Lugert et al. reveal a striking degree of astroglial diversity in the relatively finite region comprising the SGZ. The authors observe S100B$^+$/EGFP$^+$ glia, EGFP$^+$/S100B$^-$ glia, radial Blbp$^+$/EGFP$^+$ cells, horizontal Blbp$^+$/EGFP$^+$ cells, and several morphologically distinguishable glial types that do not express these markers. Although not possible to conclude on the basis of the current data, heterogeneous glial subpopulations may just be the same population distinguished temporally by cyclical or sequential expression of the markers investigated. Interestingly, Blbp is known to be a direct target of canonical Notch signaling, yet many Blbp$^+$/EGFP$^-$ cells persist.

The authors also note a rather striking segregation of Notch signaling to Type 1 precursors and Ascl1 (aka Mash1) to Type 2 progenitors. The antagonism between Notch receptors and Ascl1 homologs is a classic phenomenon that is well conserved across metazoa. This is compelling circumstantial evidence—along with the Notch gain- and loss-of-function studies—that the initial cell fate choice of Type 1 daughter cells is an interplay between Notch (in maintaining the glial fate) and Ascl1 (committing to a more differentiated progenitor fate—perhaps in concert with Ngn2). Future work using in vivo gain- and loss-of-function experiments of proneural genes in distinct progenitor populations would be helpful to rigorously investigate the early fate choices of neural progenitors.

The rodent dentate gyrus is a robust natural model for how neurons can be added to the adult brain. In addition, there is much work suggesting that new neurons might play a role in learning and memory. The findings of Lugert et al. add greatly to our knowledge of the in vivo regulation of neural precursors in the normal, pathological, and aged brain. Furthermore, the study indicates future directions in which investigation is needed. A comprehensive understanding of the molecular controls of proliferation, cell fate, and neurogenesis will shed light on all of these processes and their evolutionary and biomedical significance.

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